Morphology and chaetotaxy of *Noorda blitealis* Walker, 1859 (Crambidae: Glaphyriinae) immatures

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Abstract: The biology of *Noorda blitealis* Walker, 1859 (Glaphyriinae: Crambidae: Lepidoptera) was studied on *Moringa oleifera* Lam. The duration of egg, larval, and pupal stages and mean life cycle (egg-to-adult) period are given. Larval chaetotaxy and the morphological features of egg, larvae and pupae are described and illustrated for the first time.

Keywords: *Noorda blitealis, Moringa oleifera,* egg, larval chaetotaxy, pupa, crochets, scanning electron microscopy, stereozoom microscopy

INTRODUCTION

Moringa (*Moringa oleifera* Lam., Moringaceae) is one of the most popular vegetables in southern India, with India being its largest producer globally (Saha & Sen, 2019). *Noorda blitealis* Walker, 1859 (Lepidoptera: Crambidae: Glaphyriinae) has become an important pest on Moringa and is considered to be the most serious pest as a defoliator of annual Moringa, since it occurs throughout the year and causes extensive damage (David & Ananthakrishnan, 2004; Kumari & Kotikal, 2015). Previously, *N. blitealis* was included in the subfamily Noordinae, which was treated as a subfamily by Minet (1980) within the 'Pyralidae' (now Crambidae). Defining adult characters for Noordinae include features of the tympanal organs and male genitalia (Minet, 1980; Regier *et al*., 2012). At present, Noordinae is represented by 16 or 17 species of Old World moths, all in the genus *Noorda*, although some of these are misplaced Odontiinae (Regier *et al*., 2012; Nuss *et al*., 2018). Based on the molecular systematic study by Regier *et al.* (2012), the subfamily status of Noordinae was revised and *Noorda* was re-classified in the subfamily Glaphyriinae. A reliable higher classification is obviously essential for the organization of data and prediction of traits in economically and scientifically important groups of insects, but in some cases adult morphology may provide insufficient variation for phylogenetic analyses, particularly in the Crambidae (Solis & Maes, 2002). Currently, although adult morphology has been relatively well-studied in Crambidae, taxonomic studies of immature stages are needed. Features of the immatures might prove to be a useful tool for identification and help to resolve some of the prevailing phylogenetic problems. Here, we describe the immature stage morphology of *Noorda blitealis*, which has not previously been described or illustrated, with special reference to the egg surface, larval chaetotaxy and pupal morphology.

MATERIALS AND METHODS

The study was conducted in the Insect Biosystematics Laboratory, Dept. of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu from September 2017 to February 2018. Voucher specimens are deposited in the Department of Entomology, Tamil Nadu Agricultural University, Coimbatore, India. The specimens were identified by rearing the adult from larvae in the lab (Fig. 1A, inset). More than three generations were reared, and adult vouchers were identified using Amsel (1965).

Larvae of *N. blitealis* were collected from Coimbatore (11.012° N, 76.93° E), Dharapuram, Tamil Nadu (10.7329° N, 77.5218°90 E) and Thrissur, Kerala (10.5276° N, 76.2144° E) on *M. oleifera* and were brought to the laboratory for rearing. Larvae were reared to adulthood on field-collected leaves of *M. oleifera* in circular plastic containers 11 cm in diameter and 8 cm high, covered with muslin cloth. Fresh leaves were provided at 24 h intervals. On pupation, pupae were transferred to petridishes (9.5 cm diameter) provided with cotton and placed in adult emergence cages (30 × 30 × 30 cm). On emergence, adults were released into oviposition cages (30 × 30 × 30 cm) in a 50:50 sex ratio. Shoots of *M. oleifera* were placed in a cotton-plugged conical flask with water. Honey solution (10 %) was provided as adult food. Eggs were observed to be laid on the ventral surface of leaves. On emergence, larvae were fed with fresh leaves of *M. oleifera*. To permit study of the immature stages, a continuous culture was maintained at 28±2° C and 75-80 per cent RH.

Egg morphology: From the insect culture, ten eggs were preserved in 70% ethanol in 1.5 ml microtubes and stored at 4°C. The eggs were examined under stereomicroscope (Leica M 205 C) and were assessed for the following characters: diameter of the eggs; whether the eggs were laid singly or in groups; if they were covered with hairs or were naked; the

overall shape of the egg; the number of micropylar rosette cells; the form of the micropylar rosette cells; the form of the cells in the micropylar zone; the number of first- and second-order ribs; the relative heights of the ribs and cross striae; whether the height of the ribs was constant, and the coloration of the egg. Egg terminology follows Peterson (1964).

Larval morphology: The first and last instar larvae were used for study of chaetotaxy and the setal maps were prepared for the thorax and abdomen of the larvae and post-processed using Adobe Photoshop ®. The terminology follows Hinton (1946) and Stehr (1987). First and last instars were killed by immersion in boiling water for one minute and were preserved in 70% ethanol in labeled screw-capped glass vials. Photographs of the larvae were taken with a stereomicroscope (Leica M 205 C).

Pupa morphology: Except for the larvae used for chaetotaxy studies, the remaining larvae were reared to pupal stage. The pupae were preserved in 70% ethanol in labeled screw-capped glass vials, although fresh pupae were preferred for the study. Morphological examination was done using stereomicroscopy, as above. The morphological features of the pupae were studied and pupal terminology follows Mosher (1916).

Scanning Electron Microscopy: Scanning electron microscopy (SEM) was also used to examine the eggs, using the facility at the Tamil Nadu Agricultural University, Coimbatore. Initially, preserved eggs were cleaned with a brush and air-dried. After drying, the eggs were mounted on aluminium stubs with adhesive tape and sputter-coated for three minutes with gold in a Polaron SC 502 Sputter Coater. Images were captured in SEM (Quanta 250, FEI) and the characters examined under stereomicroscope were also examined under SEM.

RESULTS

Biology (Table 1): The mean durations of egg, larval and pupal stages were 2.85±0.35, 11.47±2.75 and 6.50±0.70 days respectively. The mean life cycle (egg-to-adult) period was 26.82±4.05 days.

Table 1. Duration of immature stages of *Noorda blitealis* Walker, 1859.

Developmental stage	Duration (days)*	
	Range	Mean ± S.D
Egg	2-4	2.85±0.35
Larvae		
I instar	1-3	1.45±0.45
II instar	2-3	2.35±0.35
III instar	2-3	2.45±0.40
IV instar	1-3	1.92±0.98
V instar	2-3	2.04±0.75
Pupa	6-7	6.50±0.70
Adult longevity	5-6	5.50±0.25

*Mean of 10 observations

Egg (Fig. 1A,B,C): The eggs are circular to oblong in cross-section and creamy yellow in color with a mean diameter of 0.54±0.10 mm and length 0.38±0.25 mm. Eggs were laid singly or in groups overlapping each other on the lower surface of the leaf. SEM images show that there are hexagonal markings on the chorion of the eggs. The surface is transparent with irregular hexagonal patterns throughout (Table 2).

Larvae: There were five larval instars, confirming earlier reports (Honnalingappa, 2001; Selvi & Muthukrishnan, 2011; Kumari & Kotikal, 2015). The mature larva was 15-25 mm long, with a green body with large, similarly colored pinacula. The first instar larvae were pale green with a small, prominent, dark head and enlarged prothorax covered with numerous setae. The larvae were minute and hence barely visible to the naked eye. The color of the larvae changed from pale green to yellowish to pinkish before pupation. The mean duration of the first instar larva was 1.45±0.45 days. The fifth instar larva had a duration of 2.04±0.75 days. The last instar larva was 15–18 mm long, and was stout and light pink in color. The larvae lost its color on boiling for preservation. A setal map is depicted in Fig. 2. Larvae fed on and defoliated the leaves.

Table 2. Characteristics of the eggs of *Noorda blitealis* Walker, 1859.

Egg character	Description
Mean diameter of the eggs	0.38 ± 0. 25 mm (Mean ± SD)
Eggs were laid singly or in groups	Group
Covered with hairs or naked	Naked
Shape of the egg	Oblong
Markings in chorion	Hexagonal markings present
Color of egg	Creamish yellow
Pattern of cells in the micropylar zone	Not visible

Head (Figs. 3A,B): The cranium is sclerotized, light brown to brownish yellow, the median epicranial suture is very short, one third the length of the lateral adfrontal suture. The frontoclypeus is long and narrow. The epicranial suture and lateral adfrontal area are well marked. The stemmatal area is not well differentiated from rest of the cranium, with six stemmata. The fifth stemma is present at the base of the antenna and the sixth is present behind the fourth stemma. Each half of the head bears 17 tactile setae, 4 proprioceptors and 10 pores. The frontal group is unisetose with setae F1 and pore Fa, F1 closer to lateral margin of the frons, posterad to C1. Fa is present near the median longitudinal line, anteromesad to F1. The clypeal group is bisetose with C1 and C2, with C1 close to epicondyle, and shorter than C2. The adfrontal group is bisetose with setae AF1, AF2 and pore Aa. AF2 is shorter than AF1, and AF1 is anterior to AF2. Pore AFa is anterior to AF2. The anterior group is trisetose with setae A1, A2, A3 and pore Aa: A1 is at the same level as stemma 3; A2 is posterior and in a straight line with A1; A3 is posterior and mesad to stemma 2. In length, A2 is greater than A1, which is greater than A3. Pore Aa is closer to A2 than A1. The posterodorsal group is bisetose with setae P1 and P2, along with pore Pb: P1 is longer than P2; P2 is posterolatrad to P1; Pore Pb is anterad to P2. Seta L1 and pore La represent the lateral group: L1 is dorsad to stemma 1; Pore La is mesad to L1. The stemmatal area has setae S1, S2, S3 and pore Sa, Sb: S1 and S2 are caudal to stemma 1; S3 is posterad to stemma 6; pore Sa is positioned dorsocaudal to stemma 6 and pore Sb is in front of stemma 3. The genal group consists of setae MG1 and pore Mga: MG1 is lower and towards the rear portion of the head. The microdorsal group has setae MD1, MD2, MD3 and pore Mda: MD1 is dorsolaterad to P2; MD2 lies in between MD1 and MD3, the latter lateral to MD2; pore MDa is closer to MD2 than MD3.

Thoracic chaetotaxy: *T1* (Fig. 3B)*:* The prothoracic shield is slightly elongated, less sclerotized, with the anterior margin flattened with dark patches. An elongated dark patch is present in the middle of shield, which is a characteristic feature of this species. The prothoracic plate also has small brown dots near the mid-dorsal line on which D1 arises, and both halves of the plate have six setae. The XD group is near the anterior margin of the shield; XD2 is anteroventrad to XD1, and XD1 is longer than XD2. The dorsal group is bisetose with D1 present close to the mid-dorsal line: D2 is near the posterior margin of the shield, posteroventrad to D1, and D2 is longer than D1. The SD group is bisetose near the posterolateral margin of the shield: SD1 is longer and ventral to XD2, and SD1 is longer than SD2. The lateral group is bisetose and prespiracular in position with L1 and L2 on the same pinaculum, with L1 longer and posteroventrad to L2. The subventral group is bisetose on an irregular pinaculum: SV1 is posterolateral to SV2, and SV1 is longer than SV2. The ventral group is unisetose with V1 present below the coxa near the mid-ventral line. *T2, T3* (Fig. 3C): The dorsal group is bisetose with setae D1 and D2 on the same pinaculum. D2 is anteroventrad to D1, and D2 is longer than D1. The pinaculum is concolorous with the body. An extra pinaculum is present anterolaterad to the SD pinacula. MSD1 is dorsad to MSD2 with both on the same pinaculum. The SD pinaculum has a dark discoloration. The sub-dorsal group is bisetose with setae SD1 and SD2 on a common pinaculum. SD2 is posterodorsad to SD1, and SD2 is longer than SD1. The lateral group is trisetose with setae L1 and L2 on the same pinaculum and seta L3 on different pinaculum situated posterior to L1 and L2. In length, L1 is greater than L2, and L2 is greater than L3. L1 is posteroventrad to SD1 and anterodorsad to L2; L3 is posterolateral to L1. The sub-ventral group is unisetose with seta SV1

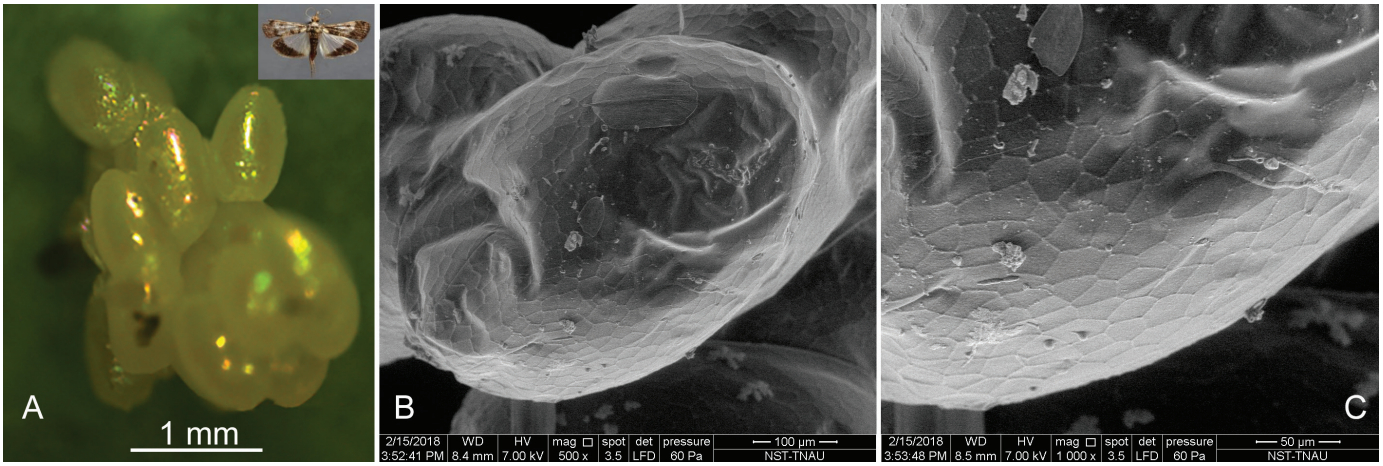

Figure 1. *Noorda blitealis*, eggs and adult. **A**. Group of eggs (adult inset top right). **B,C**. Scanning electron microscope image showing egg chorion with reticulate markings.

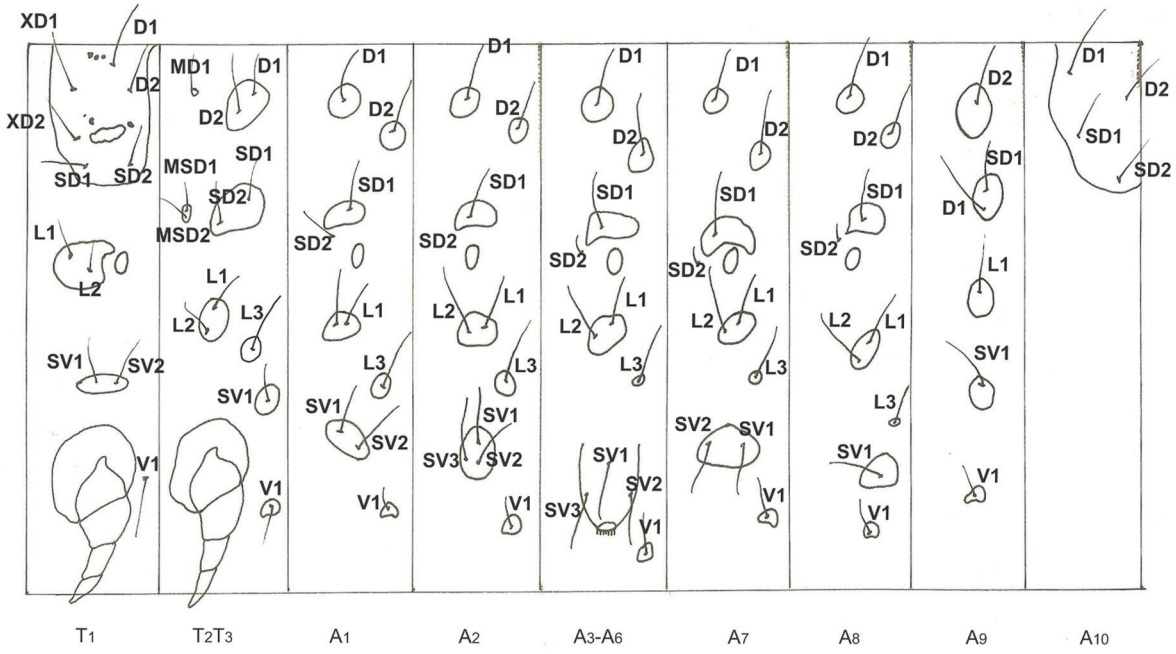

Figure 2. *Noorda blitealis*, setal map of thorax and abdomen.

on an irregularly shaped pinaculum: SV1 is ventrad to L3; MV1 is present (although difficult to see) and anterolaterad to the SV pinaculum. The ventral group is unisetose with V1 below the coxa near the mid-ventral line on a small pinaculum.

Abdominal chaetotaxy: In the first abdominal segment (Fig. 4A), D1 is anterodorsal to D2 on a separate pinaculum and it is shorter than D2. The subdorsal group is bisetose with setae SD1 and SD2: SD1 is dorsal to the spiracle; SD2 is microscopic; SD1 is anterolaterad and longer than SD2, and the position of SD2 is anterodorsal to spiracle. The lateral group is trisetose with setae L1, L2 and L3: L1 and L2 are on the same pinaculum, and L3 is on a different pinaculum; L1 is ventral to the spiracle; L2 is anteroventrad to L1; L3 is posteroventrad to L1 and L2. The subventral group is bisetose with setae SV1 lying posteroventrad to SV2: SV1 is longer than SV2; V1 is present near the mid-ventral line. In the second abdominal segment D1 is anterodorsal to D2 on a separate pinaculum and it is longer than D2. The subdorsal group has setae SD1 and SD2: SD1 is dorsal to the spiracle; SD2 is microscopic; SD1 is anterolaterad to SD2. The lateral group is trisetose with setae L1, L2 and L3: L1 and L2 are on the same pinaculum; L3 is on a different pinaculum; L1 is ventral to the spiracle; L2 is anteroventrad to L1; L3 is posteroventrad to L2. The subventral group is trisetose with seta SV1 posteroventrad to SV2: SV2 is anteroventrad to SV3. The ventral seta V1 is anteroventrad to SV1. In A3 to A6 (Fig. 4B) the dorsal group is bisetose with setae D1 and D2: D1 is anterodorsad to D2, and longer than D2 on a separate pinaculum. The subdorsal group is bisetose with seta SD1 dorsal to the spiracle and posteroventrad to D2. SD2 is microscopic and is present anterodorsad to the spiracle. The lateral group is trisetose with setae L1 and L2 present on the same pinaculum; L3 is on a small round pinaculum. The L pinaculum is posteroventrad to the spiracle. L2 is anteroventrad to L1; L3 is posteroventrad to L1. In length, L3 is greater than L1, and L1 is greater than L2. The subventral group is trisetose with setae SV1, SV2 and SV3 on the same pinaculum dorsal to the proleg: SV1 is anterodorsad to SV2; SV3 is posteroventrad to SV2; V1 is present near the mid-ventral line. On the seventh segment (Fig. 4C) the only difference from the above segments is in the SV group. The SV group is bisetose with SV2 slightly longer than SV1. SV1 and SV2 form a straight horizontal line, and SV1 is ventral to L3. On the eighth segment (Fig. 4D) the SV group is unisetose, SV1 is ventral to L3, and V1 is ventral to SV1. Dark, elongate discontinuous SD lines are seen on the eighth and ninth segments. On the ninth abdominal segment (Fig. 4D) the dorsal group is bisetose and D2 is dorsal to D1. The subdorsal group is unisetose with setae SD1 ventrad to D1; SD1 and D2 are on on the same pinaculum. The lateral group is unisetose with L1, which is posteroventrad to SD1; SV1 is ventrad to L1. Seta V1 is close to the ventral meson. The spiracle is twice as large as on the other segments. On the tenth abdominal segment (Fig. 4D), the anal shield is not well sclerotized with dark patches, the anterior margin is flattened and the posterior margin is curved. The dorsal group is bisetose with setae D1 and D2: D1 is anterad to D2, and D1 and D2 are of almost the same length; D2 is on the distal margin of shield. The subdorsal group is on the lateral margin of the

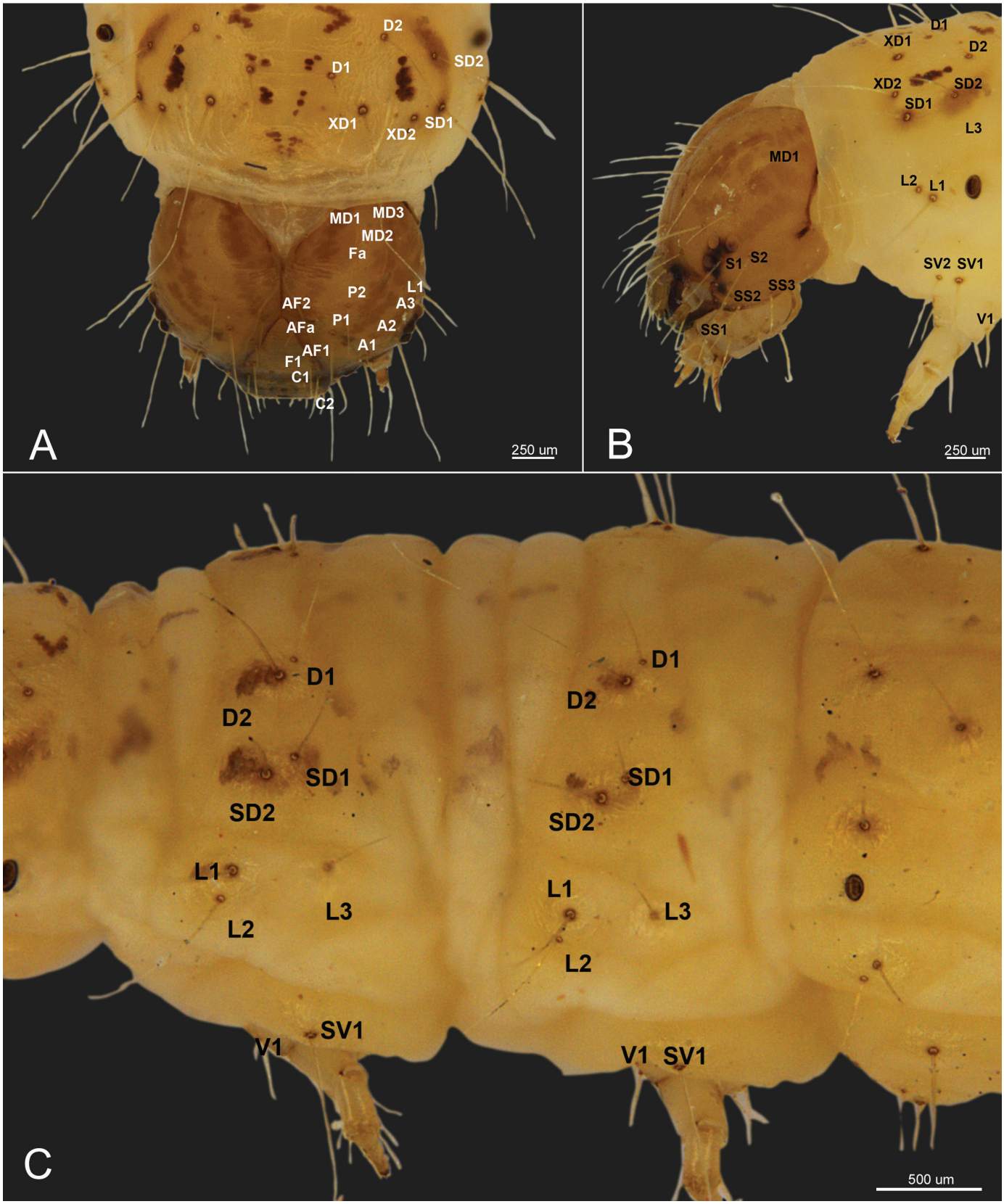

Figure 3. *Noorda blitealis*, larva, head and thorax. **A**. Dorsal view of head. **B**. Lateral view of head and T1. **C**. Lateral view of T2, T3.

shield, with seta SD1 present anteroventrad to D2; SD2 is anterolaterad to SD1. The crochets (Fig. 4E) are triordinal mesopenellipse forming an incomplete circle on abdominal segments A3-A6 and A10.

Pupa (Fig. 5A): The pupa is obtect and brown, and it was attached to the walls of the petri dish. The color turned from light brown to dark brown before moulting to adult, and the pupa had a mean length of 10.25±0.05 mm. The head is wrinkled, except for smooth ocular caps. The antennae arise from the dorsal margin of the compound eyes up to the tip of the wing pad ventrally. The antennae are parallel to each other at the meson, which is a character peculiar to Crambidae. The pilifer is present which is the characteristic feature of superfamily Pyraloidea and butterflies only. The abdominal dorsum is smooth. The wings extend beyond the fourth abdominal segment, again a characteristic

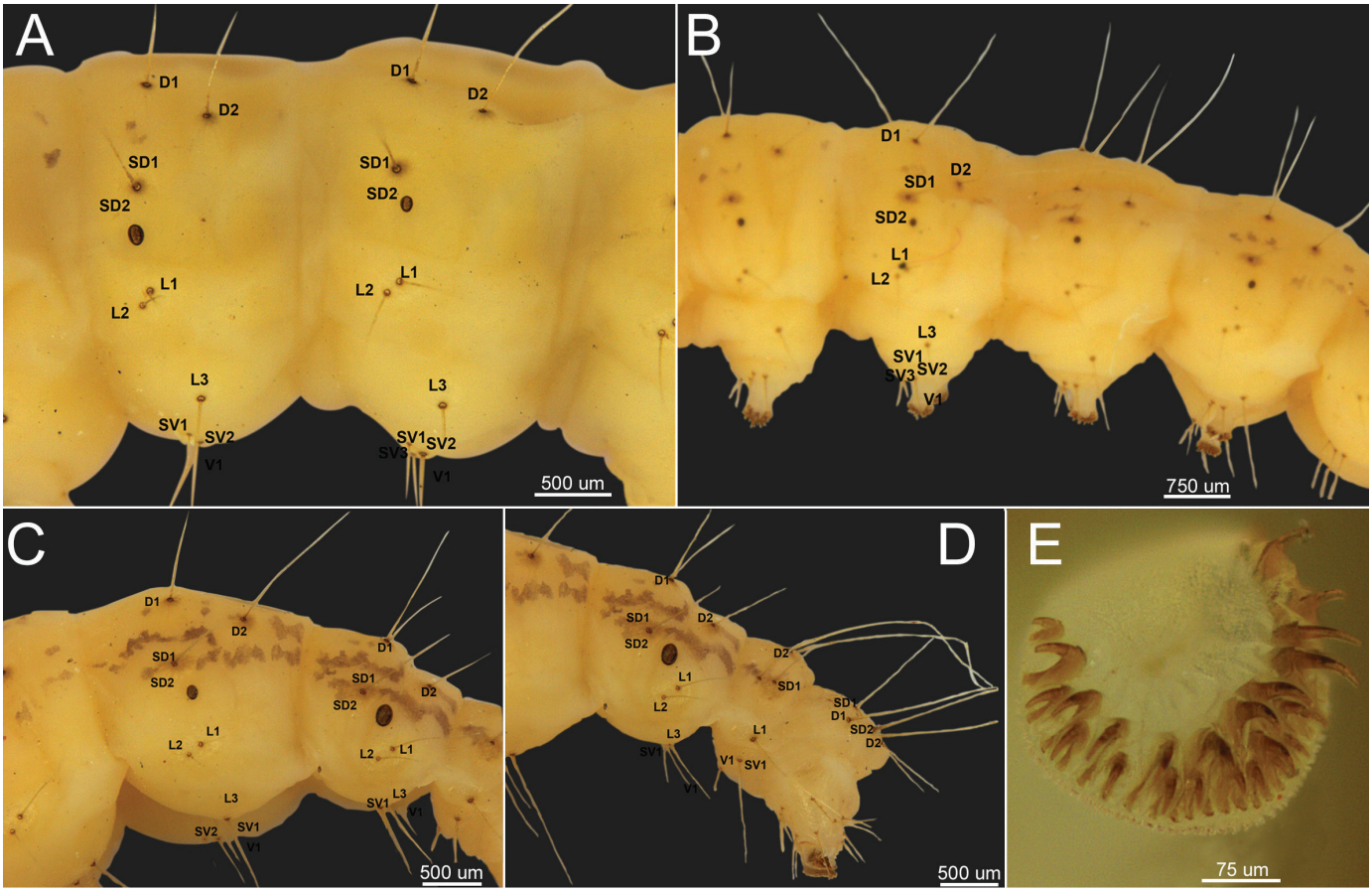

Figure 4. *Noorda blitealis*, larva, andomen. **A**. Lateral view of A1, A2. **B**. Lateral view of A3-A6. **C**. Lateral view of A7, A8. D. Lateral view of A9, A10. E. Crochets.

feature of Crambidae. The larval exuviae is smooth. The labial palpi on either side of the mid-ventral line are not prominent, a feature peculiar to the family Crambidae which was also observed in the present study. The prothoracic femur is exposed. The abdominal segments are not movable. The maxillae extend to the tornal margin of the wings. The maxillae and proboscis are long and extend toward the wing axis. Dorsally, all three thoracic segments are visible, while ventrally they area concealed by appendages (Passoa, 1988). Dorsally ten and ventrally six abdominal segments are visible. The pupal cremaster is blunt with no setae, but with two small needle-like projections (Fig. 5B). The metathoracic legs extend to the fifth abdominal segment. The frontal setae are thin. The mesothoracic spiracle is present. The posterior spiracles are sub-equal in diameter throughout the abdomen and have hidden mesothoracic and metathoracic coxae. The metathoracic legs have obtect appendages.

DISCUSSION

The mean life cycle (egg-to-adult) in this study was 26.82±4.05 days, while a previous study on the biology of *N. blitealis* reported the mean life cycle to be 18.3 days (Kumari & Kotikal, 2015). Another previous study conducted by Satty *et al.* (2013) stated that insect took around 17 days from egg to adult stage. These differences in life-cycle may perhaps be attributed to the change in location and weather conditions. The occasional placement of eggs in groups that we observed may perhaps increase protection from predators and parasitoids. Kumari & Kotikal (2015) reported that the eggs were round, either laid singly or groups of 4-7 on the ventral surface of tender leaves.

The frontal setae are thin in all subfamilies of Crambidae except Acentropinae, as described by Passoa (1985), and in several described Musotiminae, as stated by Nakamura (1977). Among Crambidae, all subfamilies except Acentropinae have a mesothoracic spiracle. Crambid pupae also have posterior spiracles sub-equal in diameter throughout the abdomen and have hidden mesothoracic and metathoracic coxae, and the metathoracic legs have obtect appendages (Passoa, 1988). All these characters are also clearly seen in the pupa of *N. blitealis*.

Pyraloidea larvae have three subventral setae present on abdominal segments three to six as stated by Solis & Maes (2002), which was also evident in the larvae of this present study. All segments lack a sclerotized ring at the base of SD1 and the L group on A9 is unisetose, which is a characteristic feature of Crambidae also evident in the larvae of the present study. As in the present study, the characteristic feature of subfamily includes the larval prothoracic shield with a prominent dark elongated spot, pore present between XD1 and XD2 in the shield, SV group bisetose in meso- and metathoracic segments, and SV group bisetose in the first abdominal segment, whereas it is trisetose in the second abdominal segment. The pinacula are not dark, unlike many other species in Spilomelinae.

Noorda blitealis shows some similarities and differences in comparison with previously described larvae of Glaphyriinae (as circumscribed by Munroe, 1964). In *N. blitealis*, the eggs are oblong in shape and laid in groups, with hexagonal markings. In *Hellula undalis* (Fabricius, 1781), the markings on the eggs are rectangular (Singh, 2010). In other Glaphyriinae

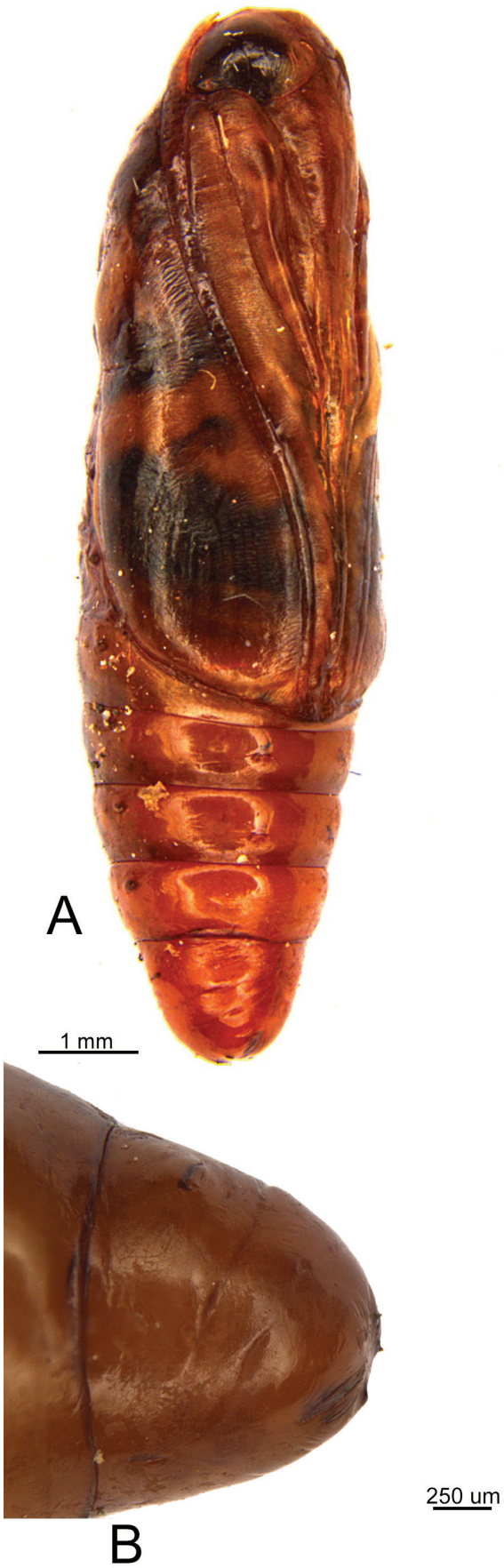

Figure 5. *Noorda blitealis*, pupa. **A**. Ventro-lateral view of pupa. **B**. Pupal cremaster.

larvae, D1 is always longer than D2 as stated by Passoa (1985), whereas in *Noorda* it varies, but mostly D2 is longer than D1 on most segments. *Noorda blitealis* larvae have a bisetose SV group in A1, and the V1 pinaculum is circular, which has been earlier reported only in *H. undalis* and *Hellula phidilealis* (Walker, 1859) and is characteristic of Glaphyriinae (Passoa, 1985). The presence of dots and markings on the prothoracic shield is again a common character in the subfamily. In larvae of *Dicymolomia metalliferalis* (Packard, 1873), a case-bearing Glaphyriinae, the prothoracic plate shows the same character (Wagner, 1985). Also, the arrangement of crochets are similar in *D. metalliferalis, H. undalis and N. blitealis* (Wagner, 1985). The pupae of *N. blitealis* have maxillary palps absent, which has been previously reported as a distinguishing feature of Glaphyriinae (Passoa, 1985). The above characters all support the placement of *N. blitealis* in Glaphyriinae. Nevertheless, there are many features that differentiate *N. blitealis* and *H. undalis*, including the following in the larvae of *N. blitealis*: (i) a dark brown elongated spot is present near the posterior edge of the prothoracic shield dorsad to SD1 and SD2; (ii) small black specks are present near the mid-dorsal line on the prothoracic plate; (iii) all the stemmata are of similar size; (iv) the head is reddish brown, and P1 and P2 are in a straight line with P1 longer than P2 with a longer median adfrontal line; (v) the crochets are clustered with the inner circle of crochets becoming longer and darker. In the larvae of *H. undalis*: (i) small, hyaline markings are present on the prothoracic shield above SD1 and SD2; (ii) small specks are absent on the prothoracic shield; (iii) stemma one is relatively larger than the rest; (iv) the head is yellowish cream, and P2 is anterodorsad to P1 with the lateral adfrontal line much longer compared to the median line; (v) the crochets are not clustered, and are of uniform color throughout.

Recent molecular studies of pyraloids have helped to resolve the relationships within this superfamily (Regier *et al*., 2009; Mutanen *et al.*, 2010; Cho *et al.*, 2011). Future integrative approaches should combine information from the immature stage morphology with adult morphological and molecular studies.

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CORRIGENDA

The printed version of this article (DOI: 10.5281/zenodo.2847700) replaces the original electronic version of this article published online on 3 May 2019 (DOI: 10.5281/zenodo.2653730). The following corrections were made to the original version:

1. Figure 5B: The image was replaced.
2. Page 49, pupa description: The following sentence "The pupal cremaster is blunt with no setae, but with two small needle-like projections (Fig. 5B)" replaced the original two sentences "The pupal end is blunt, unlike the pointed end seen in Spilomelinae. The six pupal cremaster setae are very minute, thin, spirally curved in the terminus at different heights (Fig. 5B)."

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